STUDIES ON THE BLOOD OF INSECTS.

I. The Composition of the Blood.*

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1. *Introductory.*

In studying the blood of insects in connection with respiration and coagulation or clotting, the writer found it necessary to make rather extensive studies of the various structural elements of the blood and the composition of the plasma. Certain phases pertaining to respiration have been published in part (1921). In this paper some of the results on the composition of the plasma are presented, intended as a preliminary report on the subject, since the investigations are far from complete. To summarize our knowledge on this topic, I have included the results of other investigators, crediting these at the respective places.

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2. *Reaction and Volume.*

The reaction of insect blood is slightly alkaline (Miall and Denny, 1883) or neutral to moist litmus paper. In *Leptinotarsa, Dytiscus,* and *Hydrophilus,* for instance, it is neutral to litmus in the adults, alkaline in the larvae; but distinctly alkaline in the adults to more sensitive indicators and various stains, such as hematoxylin and methyl violet. After death, or even before, the blood changes to acid. Thus, the blood of sluggish *Enallagma* and *Aeshna,* specimens so weak that they barely moved a leg, was found to be distinctly acid to litmus paper and Congo red. The

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acidity is probably due to the large amount of carbonates in solution or gathered in loose saccules, which crystallize out spontaneously on exposure to the air. This acidity increases markedly a few hours after death.

To the touch insect blood is very viscid and gelatinous. This is due to the gelatin and fibrinogen present and perhaps to some extent to the albumins and globulins. The viscosity increases greatly with the alkalinity. To the taste somewhat diluted blood is distinctly salty, although occasionally bitter, or even acrid.

The volume of the blood varies according to the stage of life (Landois, 1864) and the period of feeding. It is greatest in the pre-pupal period in holometabola, or the pre-imaginal period in hemimetabola, and smallest, proportionately, in adults. In Vertebrates the approximate proportion is about one volume of corpuscles to two volumes of plasma. In five c.c. of centrifuged oxalated blood taken from full-grown but starved *Leptinotarsa* larvae, the bulk of corpuscles formed about one eightieth of the total, hence a proportional relation of approximately one fortieth, since the oxalate solution formed half of the volume. In fully fed specimens the proportion is even less, about one sixtieth, while in adults the number of corpuscles is decreased so markedly that an estimate of one one-hundredth does not seem exaggerated. It is difficult to make numerical counts, since the gelatin and almost immediate fibrin formation prevent thorough mixing with the usual fluids.

After feeding the volume of plasma increases greatly, distending the haemocoel, so that larvae appear turgid. If starved, the integument becomes flaccid and wrinkled, indicating a decrease in volume of plasma. There is no decrease, however, in the number of corpuscles, as indicated by the experiments noted. The volume of plasma appears to have little relation to growth or transformation. I have kept *Leptinotarsa* larvae for two weeks, until they were shriveled to apparently half their normal size. They pupated and emerged practically full sized, this despite the plausible assumption that with the loss of plasma a portion of the reserve food supply had been used up in the period of starvation.

3. *Color of the Blood.*

As a rule, the blood has some slight tinge of color. I have found the blood clear, slightly tinged with yellow, yellow, orange,

orange-red, red, bluish, blue-green, and deep green, confirming the colors found by various investigators. The following list shows the colors in a number of insects examined personally, together with the type of food eaten:

Name.	Stage.	Color of Blood.	Type of Food.
Perloidea			
Pteronarcys sp.	larva	orange-red	mixed
Pteronarcys sp.	adult	orange-red	nectar
Acroneura sp.	nymph	yellow	animal
Acroneura sp.	adult	yellow	??
Ephemeroidea			
Ephemera sp.	nymph	clear	mixed
Mountain species.	nymph	red	mixed
Odonata			
Enallagma sp.	nymph	green	animal
Ischnura sp.	nymph	blue-green	mixed
Aeshna sp.	nymph	green	animal
Anax sp.	nymph	green	animal
Libellula sp.	nymph	green	animal
Hemiptera			
Notonecta sp.	nymph	green	animal juices
Notonecta sp.	adult	yellowish	animal juices
Gerris sp.	adult	yellow	animal juices
Belostoma sp.	nymph	green	animal juices
Belostoma sp.	adult	greenish	animal juices
Orthoptera			
		yellow	
Ceuthophilus sp.	adult	yellow	mixed
Gryllus sp.	adult	faint yellow	plant
Locustidae spp.	adults	clear, yellowish	plant
Microcentrum sp. . . .	nymphs	yellowish	plant
Trichoptera			
Phryganea sp.	larva	clear	plant
Leptocerus sp.	larva	yellow	mixed
Lepidoptera			
Mamestra sp.	adult	clear	nectar
Clisiocampa sp.	larva	green	plant
Deilephila sp.	larva	green	plant
Pieris rapae	larva	green	plant
Cossus sp.	larva	yellow	wood
Samia cecropia.	adult	clear	nectar

Coleoptera			
Calosoma sp.	adult	yellowish	animal
Dytiscus sp.	larva	green	animal juices
Dytiscus sp.	adult	greenish, clear	animal
Hydrophilus sp.	larva	yellow, orange	animal
Hydrophilus sp.	adult	yellow	animal
Thermonectes sp.	larva	yellowish, greenish	animal
Thermonectes sp.	adult	yellow	animal
Tenebrio sp.	larva	yellow	mixed
Tenebrio sp.	adult	bright yellow	mixed
Coccinellidae spp. . . .	adults	yellow	plant
Prionus sp.	larva	yellow	wood
Prionus sp.	adult	yellow	wood
Trogoderma sp.	larva	clear	dry food
Leptinotarsa sp.	larva	orange, orange-red	plant
Leptinotarsa sp.	adult	orange	plant
Diptera			
Calliphora sp.	adult	clear	micro-food
Musca sp.	larva	yellowish	mixed
Eristalis sp.	larva	clear	micro-food
Odontomyia sp.	larva	greenish	micro-food
Stratiomyia sp.	larva	greenish	micro-food
Miastor sp.	adult	clear	flesh
Chironomus sp.	larva	red	micro-food
Protenthes sp.	larva	clear, bluish	micro-food
Various blood-sucking flies	adult	red	animal
Hymenoptera			
Tenthredo sp.	larva	faint green	plant
Vespa sp.	adult	clear	plant
Bombus sp.	adult	clear	plant

The foregoing list could be extended greatly; but my purpose is to show only the variation of color within a few of the orders. It is evident from the table that there is no correlation between the color of the blood of a species and the type of food eaten. All shades may be found in both herbivorous and carnivorous species. Of particular interest is the fact that the blood of adults is lighter in color than that of the larvae; in *Hydrophilus* and *Dytiscus* it even differs in color in the stages—greenish in the larvae, yellow or bright orange in the adults. Geyer (1913) also noted some sexual differences in certain moths, the blood of the males being clear or faintly tinged, that of the females brightly colored (*Bombyx mori, Xanthia flavago*). Geyer associates this difference with the pigmentation of the eggs. A similar difference exists in the sexes of *Dytiscus,* the female blood being bright orange, that of the males clear yellow.

Much confusion exists as to the identity of the various pigments, due in part to confusion in terminology. Palmer (1922) has recently called attention to the fact that often the same name was given to different substances or different names to identical substances. Czapek (1913) proposed the name of " chromolipoids " for all fat-soluble plant and animal pigments, which would include many insect pigments, particularly the greens, yellows, and reds, which are usually known as lipochromes and regarded as derived chlorophylls and xanthophylls. Tswett (1911) has shown that many plant and animal pigments are closely related, if not identical, and proposed the group name " carotinoids " for pigments of this type, with " carotins " and " xanthophylls " as the main subdivisions. As such " carotinoids " many of the larval pigments, especially of herbivorous larvae, must be regarded.

But Hopkins, Urech, and A. G. Mayer (1896, 1893, 1897) have demonstrated that similar pigments, namely, reds, yellows, orange, and some whites, in adult Lepidoptera are uric acid derivatives and therefore non-carotinoid. Hemoglobin, too, and hemocyanin are non-carotinoid. On the other hand, in adults of certain Coleoptera and Hemiptera the pigments have been shown to be carotinoids. At best, therefore, no generalizations are possible. Unfortunately, from the various studies it is not clear if whole specimens were used in the extraction of the pigments, or the blood alone. On the contrary, in a number of instances large quantities of whole specimens are specifically mentioned as used for extraction. Yet it is a fact that the pigments as deposited in the wings or epidermis, although originating from the blood, are elaborated substances of more complex composition, and hardly identical with those in solution in the blood. This is clearly shown by the studies on Lepidoptera. The *separate* study of the blood pigments and of the " fixed " pigments of wings and exoskeleton should materially affect our knowledge. The whole subject needs much investigation, particularly among carnivorous insects. For some reason these have been almost entirely neglected in pigment studies, although it appears to me that we should derive more accurate information from them as to the nature of insect pigments than from herbivorous species.

4. *Chemical Composition.*

Occasionally one finds crystals in drying blood, but only when the plasma is supersaturated with the crystallizing substance, as, for instance, triple phosphate in *Leptinotarsa* blood, or calcium sulphate in certain caterpillars. Ordinarily, however, the blood is in a "balanced" or "organized" condition and substances do not crystallize out spontaneously. Only a few crystals of calcium carbonate are nearly always to be found. There is a difference in dead insects, for here the blood is "disorganized," and one may find various crystals in abundance, chiefly carbonates, triple phosphates, and tyrosin, or, more rarely, oxalate and sodium chloride crystals, or products of protein decomposition such as histidine and leucine. In general, however, little information is to be obtained from spontaneous crystallization as to the constituents of the plasma. I found direct testing with reagents, ashing, and micro-distillation and sublimation satisfactory. For testing the blood was strongly diluted with distilled water, centrifuged, and the supernatant liquid concentrated by mild heat, not to exceed 40° C. The centrifuging removes the corpuscles and some fibrin and gelatin, but otherwise leaves the plasma intact.

Insect blood, like that of Vertebrates, is composed of corpuscles and plasma. Certain investigations on the corpuscles are to be treated in a separate paper; the corpuscles are therefore omitted in this study. The plasma contains serum, gelatin, fibrinogen, and various substances in solution. The last can be conveniently classified as water, gases, salts, foods—namely, the proteins, fats, and sugars—pigments, respiratory proteins, waste products, enzymes, and special substances.

The presence of water is evident from clotting, ashing, and starvation experiments. Its proportion is difficult to ascertain, but from the reduction in size of a drop of blood during drying the water should form fully three fourths of the plasma. Of gases, oxygen, nitrogen, and carbon dioxide are always present, although the latter should be regarded as a waste product. The oxygen may be in solution in the plasma, but more probably the larger part of it is bound to a respiratory protein—hemocyanin or hemoglobin

(Muttkowski, 1921). Carbon dioxide may also have some relation to these proteins, analogous to vertebrate blood.

Iron, copper, sodium, potassium, calcium, and magnesium were found in ashed plasma. These substances, except perhaps copper, form the chlorides, sulphates, nitrates, phosphates, and carbonates, the last primarily with calcium, the others varying. (Oenslager and Mayer, 1897, report iron, sodium, and potassium.) Ammonium is also present, as indicated by the formation of triple phosphate (ammonium magnesium phosphate). During feeding periods nitrites are present in abundance, after starving only nitrates.

Among organic substances, A. G. Mayer (1897) reports albumin, globulin, and fibrinogen, Cuénot (1897) hemoxanthin and fibrinogen. The latter is probably accompanied by thrombin, although its presence was not demonstrated in the material available. The plasma also contains gelatin, a nucleoprotein, and, at feeding, various hydrolyzed proteins. Respiratory proteins, namely, hemoglobin or hemocyanin, should also be noted, the latter assumed on the presence of copper. Fat droplets are always present (Kolbe, Graber, Berlese, etc.), particularly during feeding periods (Berlese, 1901). Sugar is probably present, perhaps as a glucose, but the results of my experiments were confusing.

Waste products are the carbonates primarily, formed with carbon dioxide. Urates, xanthine, and purine may be present, but have not been definitely determined, although I found them in the Malpighian tubules.

Pigments of various types are usually found, including the carotinoids, uric acid compounds, and respiratory pigments. Cuénot (1897) reports uranidine in Meloë, which oxidizes and precipitates on contact with the air. Previously Krukenberg (1882) attributed a melanotic function to uranidine in Echinoderms.

Of enzymes, various types are present. Tyrosinase is secreted by the leucocytes in clotting blood, and perhaps thrombokinase. Histolytic enzymes are indicated by the breaking up of various small tissue fragments floating in the plasma. Enzymes acting on foods are perhaps present, but not definitely determined.

Certain special substances should be noted, such as cantharidine (Cuénot, 1897), and probably other substances found in insect secretions. Loman (1887) reports iodine in the gaseous secretion of a Paussid beetle, which presupposes its presence in the blood, from which it is extracted by the glandular tissue. I have also found traces of arsenic and lead in several instances, but can not regard them as normal constituents. Both of these are cumulative poisons and it is probable that the specimens might eventually have succumbed. Still, it is possible that at least in the Coleopteron *Scobicia declivis,* which, according to Burke, Hartman, and Snyder (1922), pierces layers of lead in lead cables and may even build galleries in lead sheets, a tolerance for lead has been developed and that analyses would show the presence of lead in the blood.

Berlese holds that Arthropod blood is more akin to the lymph of higher animals. "During digestive periods the plasma surrounding the alimentary tract forms a sort of chyle, while that in the peripheral parts corresponds to the lymph." This would be true for insects if a respiratory function were denied to the blood. But elsewhere (1921) I have recorded the presence of oxygen and respiratory proteins in insect blood. In insects the blood, besides carrying food, wastes, and plastic elements, contains respiratory proteins and oxygen, and thus supplements tracheal respiration. On this basis the blood of insects is analogous to that of higher animals.

While the preceding summary offers certain qualitative results, it has the weakness of not giving quantitative estimates, which would be more conclusive in determining if insect blood is more like lymph than true blood. The difficulty in quantitative estimates lies in the small amount of material available for study. From large caterpillars—Saturniids and Sphingidae—one may obtain as much as three to four cubic centimeters, from *Dytiscus, Hydrophilus,* and *Belostoma* perhaps half a c.c. Generally, however, when specimens are to be had in abundance, they are small and yield only a drop or two, as in full-grown *Leptinotarsa* larvae. Even with blood from hundreds of specimens, after separation of the corpuscles and concentration or evaporation, the residue is minute, with substances present in infinitesimal quantities and reacting only with the most sensitive reagents. With certain modifications, I have found the texts of Chamot and Tunman of excellent use for the study of the composition of the blood.

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